SYNTHESIS AND APPLICATIONS OF MULTIFUNCTIONAL ZINC OXIDE NANOPARTICLES USING PLANT EXTRACT

Synopsis

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1. INTRODUCTION

Nanotechnology is an interdisciplinary area of science which has been growing interest across the globe in forming nano revolution. An important area in nanotechnology deals with the synthesis of nanoparticles which has encountered vast progress due to numerous applications in recent decades [1]. Nanoparticles exhibits atom-like behavior due to a high surface area, a large fraction of surface atoms and the wide gap between valance and conduction band when divided to near atomic size [2].

Currently, there is a growing necessity to improve the environmentally benign nanoparticle synthetic processes that are free from toxic chemicals in the synthesis procedure [3]. Chemical and physical synthesis may leads to the presence of some deadly chemical species adsorbed on the surface that may have unfavorable effects in medical applications [4]. In chemical mediated synthesis of nanoparticles, some toxic, substances could be used as reducing and stabilizing agent for stopping the agglomeration. To overcome this problem researcher in the field of the synthesis of nanoparticles has turned in the direction of green system [5].

The use of plant and plant tissue for reduction of metal ions has attracted significant attention with in the last 30 years. A varied range of plant species has been reported for synthesis of metal nanoparticles. In plant extracts, some biomolecules are found which can reduce metal ions to nanosized materials by a single step mediated green synthesis approaches [6]. Plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticles formation. These primary and secondary metabolites are constantly involved in the redox reaction to synthesize eco-friendly nanosized particles [7]. Several studies have shown that many of these metabolites act as both reducing and stabilizing agents and inhibit the aggregation and agglomeration of the novel metallic nanoparticles by nonhazardous means [8].

Due to the unique properties of metal nanoparticles like large fraction of surface atoms, large surface energy, spatial confinement and reduced morphology make it considerably different from the corresponding bulk materials. Therefore, design and production of materials with novel applications can be achieved by controlling shape and size at nanometer scale. Nanoparticles exhibits size and shape-dependent properties which are of interest for applications ranging from

biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. These particles also have many applicatons in different fields such as medical imaging, nanocomopsites, filters, drug delivery, and hyperthermia of tumors [9].

One of the most important criteria of nanotechnology involves in developing green and eco friendly technologies for the synthesis of various nanoparticles of various size, shape, chemical composition and controlled dispersity. Metallic nanoparticles and metal oxide nanoparticles have wide biomedical application not only due to their large surface area to volume ratio but also of their high reactivity in comparison to bulk form [10].

Many studies have reported on the wide range of metal and metal oxide NP applications, owing to their unique and wide-ranging physicochemical properties. Silver (Ag) NPs, for example, are widely used for medical, textile, food packaging Gold (Au) NPs have been employed in biomedical research, while Platinum (Pt) NPs are extensively used for industrial applications. Finally, Palladium (Pd) NPs have been used as catalysts during the manufacture of pharmaceuticals and copper oxide (CuO) NPs as antifouling agents in paints and fabrics [11].

The inorganic nanoparticles such as Silver, Gold, Copper, CuO, TiO_2 and ZnO have profound antibacterial activities. Among the inorganic nanoparticles, ZnO NPs are of particular interest because they can be prepared easily inexpensive and safe material for human beings and animals. They are commonly used in the creation of health care products. ZnO NPs has entered the scientific highlight for its semiconducting properties, unique antibacterial, anti-fungal, wound healing and UV filtering properties, high catalytic and photochemical activity [12].

Recently the trend of preparing Metal/ MO NPs using extract of various plants gained popularity. Biosynthesis of ZnO NPs by plants such as brown marine macro alga *Sargassum muticum* [13], seaweeds of gulf of Mannar [14], *Aeromonas hydrophila* [15], *Parthenium hysterophorus*,[16],*Maple leaf* [17], *Solanum nigrum* [18],*Ocimum sanctum* [19], *Ocimum basilicum* [20], *P. amboinicus* [21], *Gum tragacanth* [22], bacterial and fungal pathogens such as Ureolytic bacteria [23], *Lactobacillus plantarum* [24], *Nerium oleander* [25], *Coptidis rhizoma* [26], *Terminalia chebula* [27], *Cassia edulis* [28] and *Cuminum cyminum* [29] have been reported. Biosynthetic and environment friendly technology for the synthesis of zinc oxide ZnO NPs are believed to be

nontoxic, bio-safe, and bio-compatible. It is attractive especially for bio-medical applications such as drug carriers, cosmetics, and fillings in medical materials.

Zinc oxide, with its unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, wide range of radiation absorption and high photo stability, is a multifunctional material [30]. Zinc oxide, an important semiconductor having a direct band gap (3.37eV) and a large exciton-binding energy (60 meV) is considered as a potential material not only in optoelectronic applications but also recognized as safe and approved for biological applications. Hence it is accepted to be one of the multitasking metal oxide with unique physiochemical properties for wide applications [31]. Zinc oxide may be a distinctive material that exhibits conductive, electricity and pyro-electric properties and has versatile applications in transparent electronics, ultraviolet (UV) light emitters, electricity devices, chemical sensors, spin electronics, personal care products, coating and paints [32]. ZnO NPs also have excellent stability, robustness, biocompatibility and longer shelf life compared to other antimicrobial agents [33].

ZnO has attracted a special attention as antibacterial agent. For instance, ZnO inhibits the adhesion and internalization of entero toxigenic *E. coli* (ETEC) into enterocytes. In addition, ZnO nanoparticles (ZnO-NPs) exhibit antibacterial activity and can reduce the attachment and viability of microbes on biomedical surfaces [34]. ZnO NPs can interact with membrane, which would lead to deformation and rupture, blockage of important ion channels, penetration of extremely small sized nanoparticles that effectively interact with several cellular components and cause oxidative stress, resulting in extensive protein and DNA damage. This might lead to cell wall rupture, leakage of cellular content and finally to bacterial death [35].

Nanoparticles of semiconductors such as Titanium dioxide, Zinc oxide, Iron oxide and Cadmium sulphide have attracted extensive attention as a photo catalyst for the degradation organic pollutants in water and air. Photo catalyst is also called photochemical catalyst and the function is similar as the chlorophyll in the photo synthesis [36]. Nanosized semi-conductors act as an effective photo catalytic agent resulting from either large surface area or quantum confinement effects of charge carriers for the degradation of organic contaminants in water under UV light and Sunlight irradiation. The simplest mechanism of photo catalytic reaction is the generation of electron–hole pair on the surface of the semi-conductor and its endpoint is as follows. When a

photo catalyst is exposed to the light which possesses stronger energy than its band gap energy, then electron–hole pairs diffuse out to the surface of the photo catalyst and take part in the chemical reaction with the electron acceptor and donor. These free holes and electrons convert to the neighboring oxygen and water molecules into OH^- free radicals and superoxide's and these acts as strong oxidizing agents for the degradation of dyes [37].

Many different semiconductor materials, such as TiO_2, ZnO, ZrO_2, SrO_2, Fe_2O_3, CdS and ZnS have been used to photo-degrade different pollutants. Among these, ZnO is known to be the best photo-catalyst for the degradation of several environmental contaminants. The photo-catalytic properties of ZnO have been widely studied, because of its low cost, high photochemical reactivity and nontoxic nature [38].

Considering the enormous potential of green plants, the present work emphasizes on the synthesis of Zinc oxide nanoparticles using two important medicinal plants *Centella asiatica* and *Asparagus racemosus* and has investigated their photocatalytic and microbial activities. Centella asiatica (Fig.1) is a perennial herb of "Apiaceae" family, known as Gotu kola or Indian pennywort/ Tiger grass. These species are largely found in the Indian subcontinent, Southeast Asia and wetland regions of the US. In traditional Asian medicine, the herb of *C. asiatica* has been used for hundreds of years, particularly in dermatological conditions, to recover small wounds, scratches, burns, hypertrophic wounds healing, and as an anti-inflammatory agent, particularly in eczema [39]. It contains active constituents such as triterpenoid saponins such as asiatic acid, asiaticoside, madecassic acid, volatile oils, flavonoids, tannins and phytosterols. In Ayurvedic medicine it is used to treat nervous, blood and skin diseases apart from antioxidant, cardioprotective, anti-hypertensive, anti-cancer activities etc [40].

Similarly, *Asparagus racemosus* (Fig.2) is known as the "Queen of herbs", because it promotes love and devotion. *Asparagus racemosus* (family Asparagaceae) also known by the name Shatavari is one of the well-known drugs in Ayurveda, effective in treating madhur rasam, madhur vipakam, seet-veeryam, som rogam, chronic fever and internal heat. This herb is highly effective in problems related with female reproductive system. Charak Samhita written by Charak and Ashtang Hridyam written by Vagbhata, the two main texts on Ayurvedic medicines, list Asparagus racemosus (A. racemosus) as part of the formulas to treat women's health disorder. A. racemosus is a well-known Ayurvedic rasayana which prevent ageing, increase

longevity and impart immunity [41]. The roots of satamuli are considered alterative, stomachic, tonic, aphrodisiac and astringent to the bowels and roots are used in the treatment of kidney and liver disorders, gleet and gonorrhea, rheumatism and diseases of the nervous system [42]. Taking advantage of these medicinal species, we wanted to extend their applications to antibacterial and biological activities.

Fig.1 ***Centella asiatica*** (Gotu Kola) Leaves Fig.2 ***Asparagus racemosus*** (Shatabri) Roots

2. Literature Review

Due to the increasing popularity of green methods, different works had been done to synthesize ZnO NPs using different sources like bacteria, fungus, algae, plants and others. Plant parts like leaf, stem, root, fruit and seed have been used for ZnO NPs synthesis because of the exclusive phytochemicals that they produce.

Sangeetha et al. (2011) studied the synthesis of ZnO nanoparticles using *Aloe barbadensis* miller leaf extract which shows distinct poly dispersity and the particle size ranges from 25 to 45 nm with an average size of 35 nm. Maximum nanoparticles shows particle size of 30 nm with distinct cap, which may be due to the flavonoids, proteins, and other functional groups present in the leaf broth of aloe vera and likely to be responsible for the formation of zinc oxide nanoparticles [43].

Gunalan et al. (2012) prepared ZnO nanoparticles against bacterial and fungal pathogens and various microbiological tests were performed using varying concentrations of green and

chemical ZnO nanoparticles with sizes 40 to 25 nm respectively. It can be concluded that the ZnO nanoparticles constitute an effective antimicrobial agent against pathogenic microorganisms [44].

Nagajyothi et al. (2013) described Green route biosynthesis, characterization and catalytic activity of ZnO nanoparticles. The UV Visible absorption spectrum shows an absorption band at 327 nm due to ZnO nanoparticles. TEM analysis shows ZnO NPs particles size in the range of 8.48 to 32.51 nm. The average mean size of ZnO NPs was 21.12 nm [45].

Nagarajan et al. (2013) synthesized Extracellular synthesis of ZnO nanoparticles using seeweeds of Gulf of Mannar, India. The extracellular biosynthesized clear ZnO nanoparticles size 36 nm. Through characterization techniques such as DLS, AFM, SEM- EDX, TEM, XRD and FTIR. The biosynthesized ZnO nanoparticles are effective antibacterial agents against Gram-positive than Gram-negative bacteria [46].

Azizi et al. (2014) reported Green ZnO nanoparticles using brown marine macroalga *Sargassum muticum* aqueous extract. Pure ZnO NPs were obtained after calcinations of S.muticum formed ZnO at 450^0C. FESEM analysis shows that the pure ZnO NPs synthesized have hexagonal wurtzite structure and the average size ranged from 30 to 57 nm. According to the FTIR analysis, the polysaccharides present in the aqueous extract of S. muticum involved in the formation of ZnO nanoparticles. The ZnO nanoparticles have hexagonal structures and the particle size ranges from 30 to 57 nm with a mean size of 42 nm [47].

Nagajyothi et al. (2014) investigated antibacterial, antioxidant and cytotoxic activities of ZnO nanoparticles using *Coptidis rhizoma*. TEM images confirmed the presence of spherical and rod shaped ZnO nanoparticles in the range of 2.90 -25.20 nm. Green synthesized ZnO nanoparticles exhibited moderate antibacterial activity against Gram-positive and Gram-negative bacteria and excellent DPPH free radical scavenging activity [48].

Yuvakkumar et al. (2014) synthesized ZnO nanocrystals using Rambutan (*Nephelium lappaceum L.*) peel extract and its antibacterial applications. The prepared ZnO nanocrystals were coated on the cotton fabric and their antibacterial activities were analyzed. ZnO nanocrystals coated cotton showed good antibacterial activity towards Escherichia coli (E. coli.), Gram-negative bacteria and Staphylococcus aureus (S.aureus), Gram- positive bacteria [49].

Rana et al. (2015) described studied the photocatalytic study which shows an effective photodegradation of RhB dye by the green- synthesized ZnO NPs [50].

Anbuvannan et al. (2015) reported *Anisochilus carnosus* leaf extract mediated synthesis of ZnO nanoparticles for antibacterial and photo-catalytic activities. The photo-catalytic performance the green synthesized ZnO showed its enhanced activity against the organic dye methylene blue under UV irradiation. The prepared ZnO nanoparticles showed potential antibacterial activities against the pathogens subjected for analysis. The diameter of the cluster ZnO nanoparticles found to be in the range of 20-40 nm [51].

Suresh et al. (2015) investigated the structure and morphology which result in a hexagonal wurtize structure. UV visible absorption of ZnO nanoparticle showed absorption band gap absorption of ZnO due to the electron transitions from the valance band to the conduction band. TEM images confirm the formation of nanoparticles and the average crystallite sizes were found to be 5-15 nm. Methylene blue (MB) dye was effectively degraded under UV and sunlight illumination in the presence of ZnO nanoparticles [52].

Janaki et al. (2015) described synthesis, characteristics and antimicrobial activity of ZnO nanoparticles. The synthesized nano crystallites of ZnO are in the range of 23-26 nm [53].

Supraja et al. (2015) synthesized *Boswellia ovalifoliolata* stem bark extract mediated ZnO nanoparticles which shows good antimicrobial activity against bacteria and fungi. Maximum zone of inhibition was identified at 170 ppm when compared to 100 and 50 ppm [54].

Geetha et al. (2016) reported the structure and morphology of the samples was investigated using PXRD, UV Vis, SEM and TEM measurements. PXRD measurements showed that the particle size is between 6 nm and 21 nm which are supported by SEM AND TEM analysis. UV visible spectrum showed that as the particle size decreases energy gap increases. PL showed prominent peaks at 392 nm [55].

Lingaraju et al. (2016) investigated Biogenic synthesis of ZnO nanoparticles using *Ruta graveolens* (L.) and their antibacterial and antioxidant activities. The PXRD pattern confirmed single phase, hexagonal wurtzite structure and the crystallite size was in nano-range and the same was confirmed by TEM analysis. UV-visible absorption spectrum of the as formed ZnO

nanoparticles is blue shifted in comparison to ZnO bulk. Significant antibacterial activity was observed on S. aureus and P. demolyticum and moderate activity on K. aerogenes. E. coli,with the concentration of 200 and 400 µg per well by agar well diffusion method [56].

Mahehendra et al. (2017) described synthesis of ZnO NPs showed an absorption peak at 305 nm which is one of the characteristic features of ZnO NPs. The SEM images confirmed the agglomeration of smaller nanoparticles. The plant extract as well as bio-fabricated ZnO NPs offered significant inhibition against Gram- positive (B. subtilis and Staph. Aureus) and Gram nrgative (P.aeruginosa and E.coli) bacteria. The minimum inhibitory concentration (MIC) of bio-fabricated ZnO NPs and plant extract was found between 4.8 to 625 g/ml against test pathogens, which was valid with live and dead cell analysis [57].

Murali et al. (2017) synthesized ZnO NPs from the leaf extract of *Ceropegia candelabrum* using a simple hydrothermal process. The synthesized ZnO NPs showed an absorption peak at 320 nm. The FTIR characterization revealed a spectrum band at 551.93 cm corresponding to the functional group metal oxide. XRD results are in corroboration with SEM images as the synthesized particles were of hexagonal wurtzite shape and the size of the particle were in the range of 12-35 nm calculated using Scherrer's formula. The elemental analysis using EDS confirmed high zinc content of 70.48%. The biosynthesized ZnO NPs offered significant antibacterial potential against S.aureus, B.subtilis, and E.coli and S. typhi [58].

Luque et al. (2018) reported the synthesis of ZnO nanoparticles using different amounts of *Daucus carota* extract. The ZnO surface morphology was influenced by the amount of extract used during the synthesis, showing a tendency to form spheroidal and polyhedral nanoparticles. The ZnO materials offered variations in the band gap around 3.14 and 3.16eV, which may be due to the presence of K ions in the Daucus carota extract. The photo catalytic activity for the sample with 80ml of Daucus carota extract showed a degradation of 95% MB at 120 min; an improvement when compared with similar commercially available material [59].

Aminuzzaman et al. (2018) investigated ZnO nanoparticles using aqueous extract of *Garcinia mangostana* fruit pericarp as reducing agent as well as capping agent. The results showed that ZnO nanoparticles synthesized by aqueous extract of G.mangostana fruit pericarp with high purity, mostly spherical in shape with an average size of 21 nm. The extent of MG dye

degradation by measuring absorbance at its characteristics λ max value of 615 nm. ZnO nanoparticles are well- crystalline, possessing hexagonal wurtzite phase with an average size of 21 nm and exhibiting good optical properties. The photo catalytic activity of biosynthesized ZnO nanoparticles was evaluated by the photo degradation of MG dye [60].

3. Research gap Identification

A wide literature survey shows that there are few reports available for synthesis of Zinc oxide nanoparticles with medicinal plants using biological method. The present study discusses the plant mediated green synthesis of Zinc oxide nanoparticles using plant extract which is used as a reducing agent to convert the precursor into nanoparticles. It leads to the formation of controlled shape and size nanoparticles with their well-dispersed nature. Green method is advantageous such as consuming less time, low cost precursor, high purity and quantity product and not requires costly equipment and environment. In addition to this, applications of biologically synthesized NPs with a particular focus on antibacterial activity.

4. Research Objectives

Based on the previously discussed content my objectives are as follows.

1. To study the green synthesis of ZnO NPs by using different plant parts like leaf, stem roots of different medicinal plants.

2. To characterize the ZnO NPs by different characterization techniques like XRD, FTIR, SEM, TEM and UV- Visible spectroscopies.

3. To study the antibacterial and photocatalytic activity of biosynthesized ZnO NPs.

5. Proposed Methodology

In literature various chemical and physical methods are used in the synthesis of nanoparticles, which has a serious hazardous and high toxicity for living organisms. In addition, the biological synthesis of metallic nanoparticles is inexpensive and eco-friendly methods. In the present approach, leaves and roots of *Asparagus racemosus* and *Centella asiatica* plants are used as biological reducing agent to obtain Zinc oxide nanoparticles.

5.1 Materials

Zinc acetate dihydrate [Zn $(CH_3COO)_2$ $2H_2O$], Sodium Hydroxide (NaOH) and Ethanol of high purity will be used. Plant leaves and roots of Shatabari and Gotu kola will be collected from the local areas for preparation of extract.

5.2 Methods

5.2.1 Preparation of plant extract

The general protocol for the nanoparticles synthesis involves in the collection of the part of plant/ plant materials of interest from the available sites followed by washing thoroughly twice/ thrice with distil water to remove associated debris if any. Thereafter, clean and fresh plant sources will be crushed and boiled in a fixed amount of water. The resulting solution is then filtered thoroughly by using whatman filter paper until no insoluble material appeared in the broth. The filtrate then centrifuged to remove any short of solid or heavy particles present in the extract.

5.2.2 Synthesis Procedure

Metal salt solutions of required concentration will be prepared in the distilled water. A fixed volume of this solution will be taken in the round bottom flask and a fixed volume of above prepared plant extract will then be added dropwise to it with constant stirring and heating at standard parameters. The reaction will be monitored with the help of color change of the reaction mixture [61].

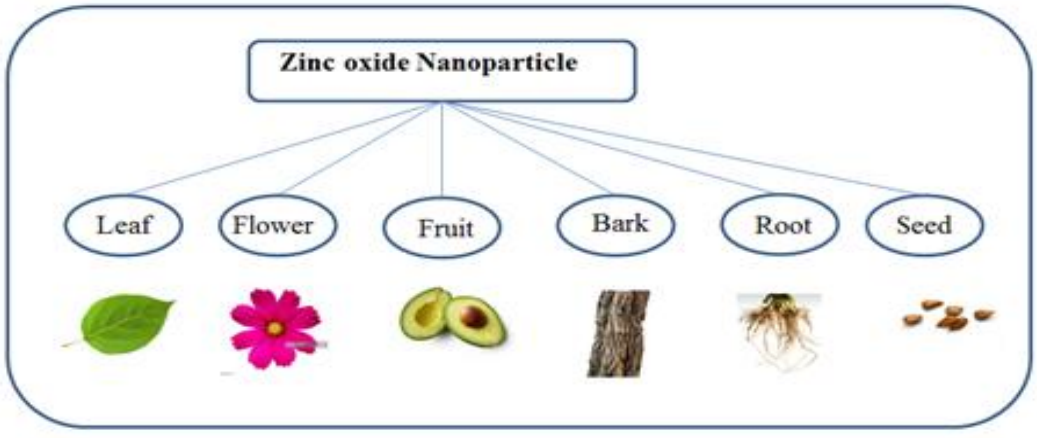

Fig :1 Synthesis of ZnO Nps from different plant sources.

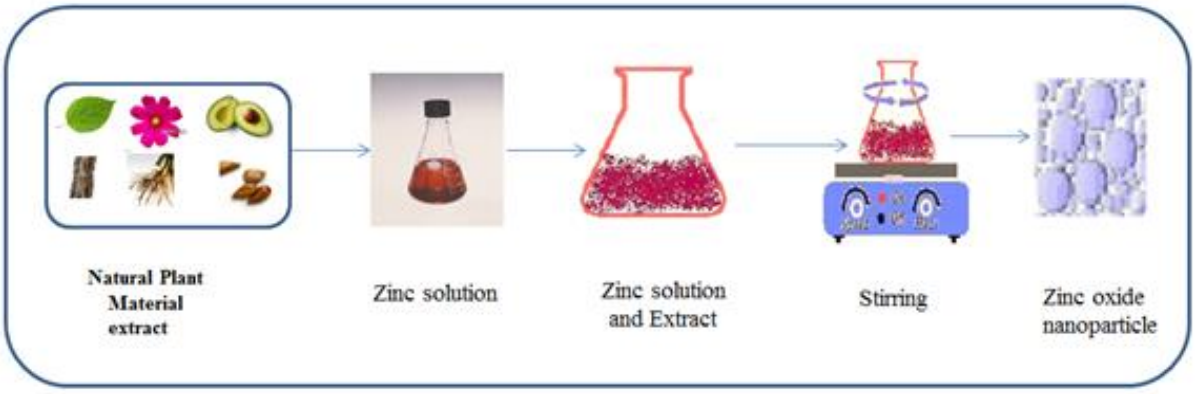

Fig: 2 Steps for the preparation of ZnO nanoparticles.

5.3 Characterization of synthesized NPs

Specifically, nanoparticle characterization is performed to assess the surface area and porosity , pore size, solubility, particle size distribution, aggregation, hydrated surface analysis, zeta potential, adsorption potential and shape, size of the interactive surfaces, crystallinity, orientation, and dispersion of nanoparticles and nanotubes in nanocomposite materials. Several techniques can be used to determine nanoparticle parameters, including, UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS) and Fourier-transform Infrared Spectroscopy (FTIR).

5.4 Antibacterial Activity

Antibacterial activities of ZnO nanoparticles will be tested with the help of well diffusion method by using Staphyloccus aureus (MTCC 737), Bacillus subtilis (MTCC 441), Pseudomonas aeruginosa (MTCC 1688), E.coli (MTCC 739). Test organisms will be collected from (IMTECH), Chandigarh. Mueller-Hinton agar will be poured in the sterilized petri plates and allowed to solidify. The plates will be left over night at room temperature to check for any contamination to appear. Bacterial strains will be spreaded on Muller-Hinton agar plates and produced the hole with the opposite side of tip. The prepared ZnO nanoparticle suspension will be added into the wells. These samples initially incubated at 37^0C for 24 hours. After the

incubation period, the zone of inhibition of each wall measured and the value was recorded, and average values were calculated for the eventual antibacterial activity.

5.5 Photocatalytic activity

Photocatalytic activity of synthesized samples will be tested on dyes by irradiating samples with light of specific wavelength.

6. Thesis Outline

CHAPTER 1 (Introduction):

This chapter will contain the brief introduction regarding NPs, biological methods, plants extract and semiconductor metal NPs.

CHAPTER 2 (Literature Review):

This chapter will deal with deep literature study of synthesis and applications of ZnO NPs.

CHAPTER 3 (Materials and Methods):

It includes the experimental details on material used, biomimitic methods of synthesis, characterization techniques and applications of synthesized NPs.

CHAPTER 4 (Results and Discussion):

This chapter will include detailed discussion on results of present work which includes the key points and various aspects of synthesis, characterization and applications of ZnO NPs.

CHAPTER 5 (Summary and Conclusions):

This chapter will summarize and correlate all the above mentioned chapters in logical manner.

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